Molecular insights into farm animal and zoonotic Salmonella infections

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Review

Molecular insights into farm animal and zoonotic *Salmonella* infections

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Salmonella enterica is a facultative intracellular pathogen of worldwide importance. Infections may present in a variety of ways, from asymptomatic colonization to inflammatory diarrhoea or typhoid fever depending on serovar- and host-specific factors. Human diarrhoeal infections are frequently acquired *via* the food chain and farm environment by virtue of the ability of selected non-typhoidal serovars to colonize the intestines of food-producing animals and contaminate the avian reproductive tract and egg. Colonization of reservoir hosts often occurs in the absence of clinical symptoms; however, some *S. enterica* serovars threaten animal health owing to their ability to cause acute enteritis or translocate from the intestines to other organs causing fever, septicaemia and abortion. Despite the availability of complete genome sequences of isolates representing several serovars, the molecular mechanisms underlying *Salmonella* colonization, pathogenesis and transmission in reservoir hosts remain ill-defined. Here we review current knowledge of the bacterial factors influencing colonization of food-producing animals by *Salmonella* and the basis of host range, differential virulence and zoonotic potential.

Keywords: *Salmonella*; colonization; virulence; animal; human; zoonoses

1. HUMAN SALMONELLOSIS AND ANIMAL RESERVOIRS

Salmonellosis in humans and other warm-blooded animals is predominantly caused by *Salmonella enterica* subspecies I. Over 2600 serovars have been classified based on reactivity of antisera to somatic lipopolysaccharide (O) and flagellar (H) antigens. From a clinical perspective, these may be broadly grouped on the basis of host range and disease presentation (Uzzau *et al.* 2000). Ubiquitous serovars such as Typhimurium and Enteritidis tend to produce acute but self-limiting enteritis in a wide range of hosts, whereas host-specific serovars are associated with severe systemic disease in healthy outbred adults of a single species that may not involve diarrhoea (e.g. Typhi in humans, Gallinarum in poultry). Host-restricted serovars are primarily associated with systemic disease in one host (e.g. Dublin in cattle, Choleraesuis in pigs), but may cause disease in a limited number of other species. Although it is convenient to classify serovars in this way, the O and H antigens alone do not dictate the outcome of infection. Thus, serovar Typhimurium is typically associated with acute enteritis in humans, cattle and pigs, yet causes typhoid in mice and colonizes the intestines of adult poultry in the absence of clinical signs. Furthermore, while *S. enterica* serovar Typhimurium (*S.* Typhimurium) infection of healthy outbred adult birds is largely restricted to the intestinal tract, oral inoculation of newly hatched chicks results in fatal systemic disease. Such outcomes are also consequent on the dose and route of inoculation, as well as the genetic background and immune status of the host. Even within serovar Typhimurium, different strains isolated from animals or humans vary in virulence for mice (Heithoff *et al.* 2008), and host-restricted variants such as pigeon-associated definitive type (DT)-2 and DT99 strains (Rabsch *et al.* 2002) exist. Similarly, non-typhoidal serovars are typically linked with enteritis in the developed world, but are increasingly associated with fatal invasive disease of infants and the immuno-compromised in sub-Saharan Africa and Asia (Gordon 2008). The extent to which this reflects the status of the hosts or possible differences among circulating strains requires further study.

Few countries report data on the societal and economic impact of salmonellosis. The FoodNet active surveillance network estimated that non-typhoidal serovars cause 1.4 million human infections in the USA each year, resulting in 168 000 visits to physicians, 15 000 hospitalizations and 400 deaths (Voetsch *et al.* 2004). Cost estimates per case range from US$50 to US$5.4 million for uncomplicated and fatal cases, respectively, at a total estimated cost of US$2.54 billion per annum

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(http://www.ers.usda.gov/data/foodborneillness/). In the UK in 2007, 14 060 laboratory-confirmed cases of non-typhoidal salmonellosis were reported, of which 52.5, 13.6 and 33.9 per cent were due to serovars Enteritidis, Typhimurium and others, respectively (http://www.defra.gov.uk/animalh/diseases/zoonoses/zoonoses_reports/zoonoses2007.pdf). The true burden of disease in the UK is likely to be far higher owing to under-reporting of illness to physicians and failure to investigate or confirm the aetiology. A survey of the worldwide distribution of serovars in human cases found *S. enterica* serovar Enteritidis (*S.* Enteritidis) to be the most prevalent in all but Central Africa, India and Central America (Ekdahl *et al.* 2005). The incidence of disease caused by different serovars varies over time and *Salmonella* evolution is further punctuated by the emergence of epidemic and multi-drug resistant variants (e.g. *S.* Enteritidis phage type (PT)-4 in humans and penta-resistant *S.* Typhimurium DT104 in livestock). The factors underlying the emergence and decline of such variants are not known. In addition to the impact of enteric disease, the global burden of typhoidal salmonellosis exerted by serovars Typhi and Paratyphi is estimated at 21.6 million cases per annum with a 1 per cent fatality rate (Crump *et al.* 2004). Owing to the theme of this volume, we have elected to focus this review on serovars associated with animal disease and zoonotic infection as typhoid in humans is associated with person–person transmission and poor sanitation.

The importance of food-producing animals as reservoirs of non-typhoidal serovars affecting humans is well-established. The pandemic of *S.* Enteritidis lasting over two decades to the present is primarily associated with poultry meat and eggs. In the UK, human cases of *S.* Enteritidis PT4 reached a peak in the early 1990s and remained stable at *ca* 25 000 laboratory-confirmed cases until 1998. Subsequent falls were coincident with implementation of live-attenuated or inactivated *S.* Enteritidis vaccines under the Lion Code of Practice issued by the British Egg Industry Council, supported by biosecurity and feed hygiene controls. This resulted in a fall in the number of UK-produced boxes of six eggs positive for *Salmonella* from one in 100 in 1995–1996 to one in 290 in 2004, albeit eggs imported to the UK retain levels as high as one in 30 boxes (http://www.food.gov.uk/multimedia/pdfs/fsis5004report.pdf). Though recent data are lacking, a UK-wide survey in 2005 indicated that 4 per cent of chicken on retail sale was contaminated with *Salmonella* (Meldrum & Wilson 2007). Although consumption of contaminated poultry meat and eggs is the primary risk factor for human non-typhoidal salmonellosis, other food-producing animals also pose a threat of zoonotic transmission. For example, serovar Typhimurium is highly prevalent in pig production, with caecal samples of 11.1 per cent of pigs testing positive for *S.* Typhimurium in a UK-wide year-long survey in 1999–2000 (Davies *et al.* 2004). *S.* Typhimurium has also been associated with lamb, beef and dairy produce, albeit low levels were recorded in the above survey.

Interestingly, such surveys reveal that some non-typhoidal serovars that are highly prevalent in food-producing animals rarely appear in humans. For example, *S.* Derby was common in UK pigs in 1999–2000 (6.3 per cent of caeca and 1.6 per cent of carcasses) yet in the same period it was rarely observed in humans even though pork products are considered to be an important food vehicle of *Salmonella* (Davies *et al.* 2004). Similarly, serovars Livingstone, Senftenberg, Agarna, Kedougou and Mbandaka were each responsible for 7–13 per cent of *Salmonella*-positive tests in chickens in 2007 (compared with 18.8 per cent for Enteritidis), yet none appear in the top 10 serovars causing human disease in the same period (http://www.defra.gov.uk/animalh/diseases/zoonoses/zoonoses_reports/zoonoses2007.pdf). The reasons why some serovars transmit efficiently *via* the food chain and elicit disease in humans are unclear and a need exists for improved diagnostic tools to predict the zoonotic and epidemic potential of isolates found in animals.

2. SALMONELLOSIS IN FOOD-PRODUCING ANIMALS

The pathogenesis, epidemiology and impact of *S. enterica* infections in food-producing animals is reviewed extensively elsewhere (Wallis & Barrow 2005). As with humans, the outcome of infection reflects both serovar- and host-specific factors and may involve enteric or systemic phases. Comparative analysis of serovars in experimental farm animal models reinforces this. For example, oral inoculation of weaned calves with serovar Dublin produces severe systemic infection, whereas *S. enterica* serovar Gallinarum (*S.* Gallinarum) is avirulent and *S.* Typhimurium elicits acute enteritis (Paulin *et al.* 2002). The systemic virulence of *S. enterica* serovar Dublin (*S.* Dublin) in cattle cannot be attributed to survival in primary macrophages, macrophage lysis (Watson *et al.* 2000*a*), damage to intestinal epithelia (Bolton *et al.* 1999), enteritis (Paulin *et al.* 2002) or to the magnitude of invasion of the ileal mucosa (Bolton *et al.* 1999; Paulin *et al.* 2002). Broadly, the same applies to the systemically virulent serovars Abortusovis in sheep (Uzzau *et al.* 2001), Choleraesuis in pigs (Watson *et al.* 2000*b*) and Gallinarum in chickens (Chadfield *et al.* 2003), compared with the broad host-range serovar Typhimurium.

A feature of some *S. enterica* infections is the development of a carrier state after primary challenge. Active carriers excrete high levels of bacteria, often in the absence of clinical signs, during recovery from enteric or systemic disease. In some cases (e.g. *S.* Dublin infection of cattle), this state may persist for life. Passive carriers are immune animals that excrete *Salmonella* acquired from a contaminated environment with no active pathology. Such animals will clear the organism if removed to a clean environment. In latent carriers, *Salmonella* can persist asymptomatically in the tissues and be excreted intermittently, for example in response to stress at parturition, transportation or mixing, as is common following *S.* Typhimurium infection of pigs. Improved understanding of the biology of carrier states is vital to control *Salmonella* in the farm environment.

Current knowledge of the molecular mechanisms underlying intestinal colonization of food-producing

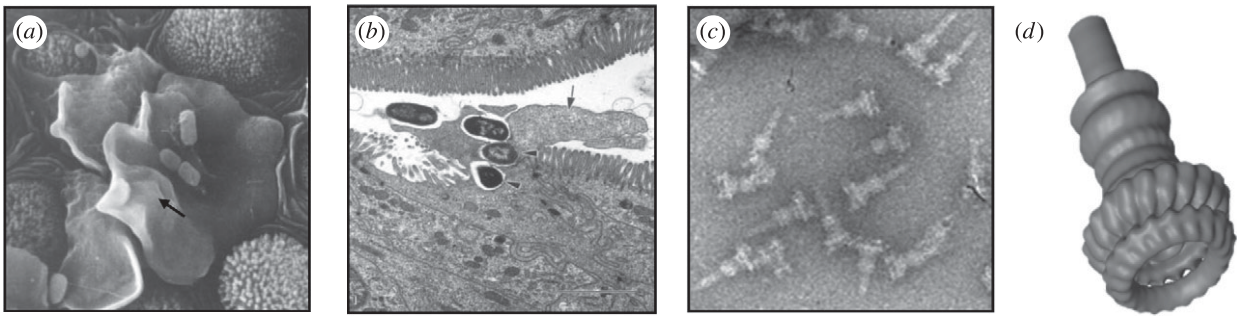

Figure 1. (*a*) Scanning electron micrograph (SEM) showing the induction of membrane ruffles by *S.* Typhimurium 20 min after inoculation of a bovine-ligated distal ileal loop. (*b*) Transmission electron micrograph (TEM) of an *S.* Typhimurium-induced membrane ruffle, shown in cross-section with bacteria residing in vacuoles (from Frost *et al.* 1997). (*c*) TEM of negatively stained needle complexes isolated from *S.* Typhimurium and (*d*) surface rendering of the reconstructed image derived from such analysis ((*d*) adapted from Marlovits *et al.* 2004).

animals, induction of enteritis, avian reproductive tract tropism and systemic virulence are reviewed below. Constraints of space preclude a detailed review of all virulence factors and their modes of action. Owing to the fact that pathogenesis varies with the serovar and host, caution is required when extrapolating the role of specific factors from one animal to another, and in particular from surrogate rodent and cell-based assays to reservoir hosts.

3. MOLECULAR BASIS OF INTESTINAL COLONIZATION OF FOOD-PRODUCING ANIMALS BY *SALMONELLA*

Despite the availability of complete genome sequences of isolates representing the key serovars afflicting farm animals, the role of the encoded genes in colonization, pathology and transmission in reservoir hosts has received relatively little study. Signature-tagged transposon mutagenesis (STM; Hensel *et al.* 1995) has markedly accelerated the assignment of roles to *Salmonella* genes *in vivo*. By this method *S.* Typhimurium transposon mutants were screened in parallel for their ability to colonize the intestines of mice and calves (260 mutants; Tsolis *et al.* 1999) and calves, chickens and pigs (1045 mutants; Morgan *et al.* 2004; Carnell *et al.* 2007). Importantly, these studies revealed that *Salmonella* deploys both conserved- and host-specific colonization factors. Many of the attenuating mutations identified by STM were located in regions of the *Salmonella* chromosome of aberrant GC content that are enriched in virulence-associated genes and mobility-related features (*Salmonella* pathogenicity islands (SPIs); reviewed in Morgan 2007).

In mice, cattle and pigs multiple independent mutations were identified in SPI-1 and -2 and defined mutants were profoundly attenuated (Hensel *et al.* 1995; Shea *et al.* 1996; Tsolis *et al.* 1999; Morgan *et al.* 2004; Carnell *et al.* 2007). SPI-1 and -2 played minimal roles in colonization of chicks by *S.* Typhimurium as determined by STM (Morgan *et al.* 2004). However, re-testing of mutants singly or in competition with the parent indicated subtle roles for both islands in persistence in chickens (Morgan *et al.* 2004; Dieye *et al.* 2009). SPI-1 and -2 encode Type III protein secretion systems (T3SSs) that are among the best characterized of all *Salmonella* virulence factors. In both ultrastructure and function, T3SSs may be likened to molecular syringes as they inject bacterial proteins (effectors) into host cells which then modulate cellular processes to the benefit of the pathogen (reviewed in Schlumberger & Hardt 2006).

The SPI-1-encoded Type III secretion apparatus (T3SS-1) plays a pivotal role in intestinal invasion by *Salmonella* by orchestrating rearrangements of the subcortical actin cytoskeleton leading to membrane 'ruffling'. Such ruffles may be observed on the apical surface of enterocytes as early as 20 min after instillation of *S.* Typhimurium into bovine-ligated ileal loops, and by transmission electron microscopy can be seen to mediate bacterial uptake into a *Salmonella*-containing vacuole (SCV; Frost *et al.* 1997; figure 1). Structural components of T3SS-1 are vital for invasion of bovine and porcine ileal mucosa by *Salmonella* (Watson *et al.* 1995; Boyen *et al.* 2006*b*; Paulin *et al.* 2007) and six effector proteins play roles in the subversion of actin dynamics (SipA, SipC, SopE/E2, SopB (SigD), SopD and SptP; reviewed in Schlumberger & Hardt 2006). Some act in concert to activate the host Rho family GTPases Cdc42 and Rac1 that promote actin assembly, either by mimicking the action of eukaryotic guanine nucleotide exchange factors (GEFs; SopE/E2) or by the generation of phosphatidyl inositol phosphates that act as secondary messengers that activate host GEFs (SopB). SipA and SipC act directly and cooperatively on actin and mediate polymerization and bundling. SptP mimics host GTPase-activating proteins to inactivate Cdc42 and Rac1 and thereby reverse SopB-, SopE- and SopE2-signalling through Rho GTPases to restore cellular architecture after invasion. While both SipA and SptP are to be found simultaneously inside *S.* Typhimurium, they are injected into cells at different times to avoid interference (Winnen *et al.* 2008). Complex interplay exists between T3SS-1 effectors (Cain *et al.* 2008) and variation in effector repertoire may lead to alterations in membrane ruffling (Perrett & Jepson 2009), and thus alter the outcome of infection. The same can be expected to apply if the secretion hierarchy is altered.

The bacteriophage-encoded *sopE* gene is not uniformly present in all *S. enterica* strains or serovars

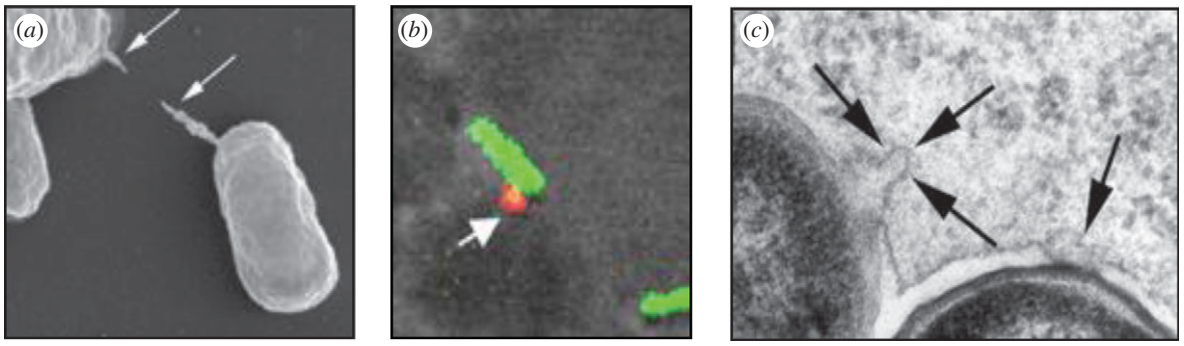

Figure 2. (*a*) SEM showing the polar T3SS-2 apparatus of *S.* Typhimurium (arrows). Such structures are absent in a SPI-2 (*ssaV*) mutant and can be labelled with specific antisera against SPI-2-encoded proteins (not shown). (*b*) Immunofluorescence micrograph showing induction of T3SS-2 (red) during macrophage infection by *S.* Typhimurium (green), (*c*) TEM of a negatively stained ultra-thin section showing connection of the bacterial and phagosomal membranes at the location of an appendage (panels *a–c* adapted from Chakravortty *et al.* 2005).

and its presence is correlated with disease in humans (Hopkins & Threlfall 2004), and the epidemic potential of *S.* Typhimurium strains from cattle (Mirold *et al.* 1999). Indeed, lysogenic conversion with the SopE-encoding bacteriophage increases the virulence of *S.* Typhimurium in calves (Zhang *et al.* 2002*a*). Analysis of the evolution of T3SSs among *S. enterica* genomes has indicated that other effectors and the T3SS proteins that translocate them into host cells are 'differentially evolved' on the basis of non-synonymous distances between the homologues (Eswarappa *et al.* 2008). Further differences exist at the level of effector expression between serovars. For example, SipC messenger (m)RNA and protein levels vary between serovar Typhimurium and Choleraesuis strains in a manner that may partly explain differences in their ability to invade porcine intestinal mucosa and induce enteritis (Paulin *et al.* 2007). Despite the role played by SPI-1 in intestinal colonization of mammals, it should be noted that strains exist that lack key functional components of T3SS-1 yet are associated with human disease (Hu *et al.* 2008).

A second Type III secretion system (T3SS-2) plays a key role in intracellular survival and replication of *Salmonella* following invasion (reviewed in Abrahams & Hensel 2006). The apparatus is ultrastructurally distinct from T3SS-1 and is deployed into the SCV membrane (figure 2). T3SS-2 modulates intracellular trafficking of the SCV to avoid fusion with lysosomes (Uchiya *et al.* 1999). It is clear that T3SS-2 also interferes with innate and adaptive immune responses, for example by impairing the NAPDH oxidase-dependent respiratory burst in macrophages (Vazquez-Torres *et al.* 2000), evading exposure to reactive nitrogen intermediates (Chakravortty *et al.* 2002), inducing caspase-1-dependent macrophage death (Monack *et al.* 2001), and inhibiting antigen-presentation by impairing peptide loading of major histocompatibility complex (MHC) molecules in dendritic cells (Halici *et al.* 2008). SPI-2 has long been associated with systemic virulence (see below), but *S.* Dublin SPI-2 mutants are also impaired in their ability to colonize the bovine intestines (Bispham *et al.* 2001), a finding supported by STM studies in calves and pigs (Morgan *et al.* 2004; Carnell *et al.* 2007). *S.* Typhimurium SPI-2 mutants are also highly attenuated in bovine ileal loops with end-to-end anastomoses incubated *in situ* for 5 days (Coombes *et al.* 2005*a*). Numerous other factors have been implicated in intracellular survival of *Salmonella*, in particular those in the PhoPQ regulon (reviewed in Kato & Groisman 2008); however, their role in livestock is unproven.

Other factors playing conserved roles in intestinal persistence include surface polysaccharides. Numerous attenuating mutations affecting lipopolysaccaharide (LPS) biosynthesis have been identified by screening random mutants in calves, pigs and chickens (Turner *et al.* 1998; Tsolis *et al.* 1999; Morgan *et al.* 2004; Carnell *et al.* 2007). However, the extent to which this is attributable to the roles of LPS in cell envelope integrity, resistance to bile salts and antimicrobial peptides or the correct insertion and folding of membrane proteins is ill-defined. For example, a mutation affecting lipid A biosynthesis (*waaN*) impaired *S.* Typhimurium virulence in calves, but also T3SS-1 function (Watson *et al.* 2000*c*). Mutations affecting the biosynthesis of colanic acid and enterobacterial common antigen were also attenuating in calves, pigs and chickens (Morgan *et al.* 2004; Carnell *et al.* 2007). Mutations affecting aspects of central intermediary metabolism (e.g. amino-acid and nucleotide biosynthesis) and selected transcriptional regulators also impaired persistence in all three hosts but the contribution of specific pathways has still to be proven.

In addition to factors playing conserved roles in intestinal colonization, host-specific virulence factors were identified by STM. For example, multiple attenuating mutations were identified in SPI-4 in calves, but not chickens or pigs challenged with the same library (Morgan *et al.* 2004; Carnell *et al.* 2007). SPI-4 encodes a Type I protein secretion system that secretes a *ca* 595 kDa protein (SiiE; Morgan *et al.* 2007) that mediates adherence (Gerlach *et al.* 2007) and invasion of bovine ileal mucosa (Morgan *et al.* 2007), possibly by working in concert with T3SS-1 (Gerlach *et al.* 2008). It is unlikely that SPI-4 acts in a fully bovine-specific manner, as SPI-4

mutants exhibited reduced virulence following oral inoculation of mice (Morgan *et al.* 2004; Gerlach *et al.* 2007).

Other adhesins that may act in a host-specific manner include autotransported proteins and fimbriae. The SPI-3-encoded fibronectin-binding autotransported protein MisL influences intestinal colonization in mice (Dorsey *et al.* 2005), chickens (Morgan *et al.* 2004) and pigs (Carnell *et al.* 2007), but was apparently not needed in calves (Morgan *et al.* 2004). Another fibronectin-binding protein (ShdA) influences *S.* Typhimurium persistence in mice (Kingsley *et al.* 2002), but plays little role in pigs (Boyen *et al.* 2006*a*). Fimbriae are proteinaceous surface appendages that mediate initial interactions between bacteria and host or abiotic surfaces. Several fimbrial loci were implicated in intestinal colonization of calves, pigs and chickens by STM, but none in all three hosts (Morgan *et al.* 2004; Carnell *et al.* 2007). Type I fimbriae have been implicated in colonization of pigs (Althouse *et al.* 2003); however the extent to which this is a direct interaction or a consequence of interference in the deployment or function of T3SSs (Field *et al.* 2008) requires investigation. A recent survey of the role of all 13 predicted major fimbrial subunits of *S.* Enteritidis PT4 identified a role for a novel locus conserved in serovars Paratyphi, Enteritidis and Gallinarum (*peg*, formerly *stc*) in chicken gut colonization (Clayton *et al.* 2008). Other fimbrial loci played little or no role in the model used. In part, this may be explained by functional redundancy as inactivation of multiple fimbrial loci has been reported to be more attenuating than single mutations in mice (van der Velden *et al.* 1998). Analysis of the fimbrial gene content of sequenced *S. enterica* genomes has not identified a single locus associated with host-specificity (Clayton *et al.* 2008); however it remains likely that they play a role in cell-, tissue- or host-tropism. With this in mind, caution is required when extrapolating findings from *in vitro* models to target hosts. This is reinforced by the finding that only one of 11 *S.* Typhimurium fimbrial subunits was expressed during culture *in vitro*, compared with nine of 11 in bovine ileal loops (Humphries *et al.* 2003).

Next-generation microarray-based methods have recently been applied to study the requirement for *S.* Typhimurium genes during systemic infection of mice (Chan *et al.* 2005; Lawley *et al.* 2006). These have the advantage of simultaneously mapping transposon insertion sites and providing a numerical measure of attenuation based on hybridization of dye-labelled cDNA flanking the insertion sites to high-density oligonucleotide arrays. The authors are applying such technology to *S.* Typhimurium in calves, pigs and chickens with the aim of assigning roles to most transposable genes in intestinal colonization. It is vital that such data are integrated with data on the content of *S. enterica* subspecies I genomes as evaluated by genome-sequencing and microarray analysis (Porwollik *et al.* 2004; Anjum *et al.* 2005), data on gene expression from transcriptome and proteome studies, and data on metabolism and regulatory networks, since this may lead to testable predictions about the basis of host-specificity. Analysis of complete genome sequences has indicated that broad host range serovars (e.g. Typhimurium, Enteritidis) maintain a wider range of intact genes than are found in serovars that are host-restricted (e.g. Choleraesuis; Chiu *et al.* 2005) or host-specific in farm animals (e.g. Gallinarum; Thomson *et al.* 2008). It is reasonable to hypothesize that the narrowing of host range in such serovars is associated with attrition of functions that are required to occupy diverse niches. Integration of data on the role of serovar Typhimurium genes in varied hosts with their conservation, sequence and expression in host-restricted serovars may indicate if this is the case. The role of serovar-specific loci and lateral gene transfer in the differential host range and virulence of serovars also requires study.

4. MOLECULAR MECHANISMS UNDERLYING THE INDUCTION OF ENTERITIS IN LIVESTOCK

Host and bacterial factors underlying *Salmonella*-induced enteritis are comprehensively reviewed elsewhere (Layton & Galyov 2007). As with invasion, structural components of T3SS-1 are required for induction of intestinal secretory and inflammatory responses in ligated ileal loops calves (Watson *et al.* 1998) and pigs (Boyen *et al.* 2006*b*; Paulin *et al.* 2007). However, it should be noted that SPI-1 mutants can still elicit inflammatory responses when incubated in ileal anastomoses for a longer duration (Coombes *et al.* 2005*a*), indicating that SPI-1-independent mechanisms exist. The extent to which invasion *per se* is required for enteritis, as opposed to the T3SS-1-mediated injection of effectors by lumenal bacteria, remains unclear. Equally invasive strains can elicit different enteropathogenic responses (Paulin *et al.* 2002) and gentamicin-protection studies indicate that most bacteria instilled into ileal loops remain extracellular (Watson *et al.* 1995). Further, cytochalasin D-mediated inhibition of *S.* Typhimurium invasion did not prevent neutrophil transmigration in a monolayer system (Gewirtz *et al.* 1999).

The T3SS-1 effector proteins SipA, SopA, SopB, SopD and SopE/E2 act in concert to induce enteritis in bovine ileal loops (Wood *et al.* 1996, 2000; Jones *et al.* 1998; Zhang *et al.* 2002*b*). SopA is a HECT-like E3 ubiquitin ligase that influences neutrophil transmigration across epithelia, possibly by ubiquitination of cellular and bacterial factors involved in inflammation (Zhang *et al.* 2006). The inositol phosphate phosphatase activity of SopB may promote enteritis by producing elevated levels of D-*myo*-inositol 1,4,5,6-tetraphosphate that in turn antagonizes the closure of chloride ion channels thereby influencing electrolyte and thus fluid secretion into the gut lumen (Norris *et al.* 1998). It is now appreciated that SopB persists long after injection into host cells and plays a role in intracellular replication by activation of cellular kinases such as Akt1 (Kuijl *et al.* 2007). SopD promotes enteritis in concert with SopB by an unknown mechanism (Jones *et al.* 1998). It remains unclear if the roles of SipA and SopE/E2 in enteritis are distinct from, or a consequence of, their roles in actin assembly and invasion. SipA appears to influence neutrophil transmigration across epithelial monolayers

by inducing apical release of the chemoattractant hepoxilin A3 in a manner dependent on protein kinase C activation (Mrsny *et al.* 2004). It is noteworthy that several T3SS-1 effectors mediate the disruption of tight junctions and thus impair intestinal barrier integrity, a process believed to be dependent on their ability to modulate cellular actin dynamics (Boyle *et al.* 2006).

As previously noted for SopE/E2 and SipC, variation in the repertoire, sequence or expression of effector proteins may explain differences in the ability of serovars to induce enteritis in animal models (Paulin *et al.* 2002, 2007). For example, SopA is truncated in the sequenced genomes of serovars Gallinarum (Thomson *et al.* 2008) and Choleraesuis (Chiu *et al.* 2005). SopA and SopE2 are absent in serovar Typhi, but the remaining enteritis-associated effectors SipA, SopB, and SopD remain active and, when exchanged for their cognate effectors, do not transfer an altered phenotype to *S.* Typhimurium in bovine ileal loops (Raffatellu *et al.* 2005). Owing to the potential for effectors to subvert cellular processes in cell-, tissue- and host-specific ways, activities detected in one system may not be relevant in others. For example, AvrA, which inhibits the pro-inflammatory NF-κB pathway in human cells *in vitro* (Collier-Hyams *et al.* 2002), does not appear to modulate early inflammatory responses in calves (Schesser *et al.* 2000; Zhang *et al.* 2002*b*). Similarly, SlrP mutants exhibit a colonization defect in mice but are not impaired of their ability to colonize the intestines of calves (Tsolis *et al.* 1999), or induce enteritis in bovine ileal loops (Zhang *et al.* 2002*b*).

Structural components of T3SS-2 are also required for induction of enteritis in calves (Bispham *et al.* 2001; Coombes *et al.* 2005*a*); however the roles of individual secreted substrates of this system are less well understood. SPI-2-encoded proteins appear to play a role in the transcytosis of flagellin subunits across epithelial cells, a process that is proposed to elicit pro-inflammatory responses by activation of basolateral Toll-like receptor 5 (Lyons *et al.* 2004). Studies in the bovine ileal loop model established a role for the *S.* Typhimurium flagella master regulator *flhD* in fluid secretion and neutrophil recruitment (Schmitt *et al.* 2001). The responses induced by a *fliC fljB* double mutant lacking flagellin structural subunits were also lower than those induced by the parent strain, but not statistically significantly so (Schmitt *et al.* 2001).

Small molecule inhibitors of Type III secretion in *Salmonella* have been described, including salicylidene acylhydrazides (Hudson *et al.* 2007; Negrea *et al.* 2007). While these can reduce the magnitude of intestinal secretory and inflammatory responses to *S.* Typhimurium in bovine ileal loops it was necessary to pre-incubate the bacteria with inhibitors in order to produce the effect (Hudson *et al.* 2007). This likely reflects the speed with which T3SS-1 is deployed and emphasizes the challenge of attaining pharmacologically active concentrations of such drugs at an appropriate location and for an adequate duration. Secreted protein vaccines also conferred limited, T3SS-1-independent, ability to protect pigs against intestinal colonization and pathology mediated by *S.* Typhimurium (Carnell *et al.* 2007).

5. MOLECULAR BASIS OF COLONIZATION OF THE AVIAN REPRODUCTIVE TRACT AND EGG

A key feature of the ongoing *S.* Enteritidis pandemic is the importance of egg contamination in transmission, the basis of which was recently reviewed (Gantois *et al.* 2009*a*). Contamination of the outer shell with intestinal contents may occur during or after oviposition and may lead to bacterial internalization during cooling of the transiently permeable shell at the point of lay (horizontal transfer). However, several lines of enquiry indicate that internal contamination of the egg is most likely to be a consequence of direct contamination of the yolk, albumen, shell membranes or shell before oviposition owing to bacterial persistence in the reproductive tract (vertical transfer; Keller *et al.* 1995; Methner *et al.* 1995; Gast & Holt 2000). In experimentally infected hens, strains of both serovar Enteritidis and Typhimurium are capable of colonizing the avian reproductive tract and the forming egg; however, *S.* Enteritidis appears to persist for longer in eggs and thus presents the greater risk of zoonotic transmission (Keller *et al.* 1997; Gantois *et al.* 2008*a*).

Improved survival in albumen is a feature of *S.* Enteritidis strains compared with serovar Typhimurium (Clavijo *et al.* 2006). Screening of transposon mutants of *S.* Enteritidis PT4 for survival in egg albumen identified 36 mutations that conferred sensitivity to albumen (Clavijo *et al.* 2006). Thirty-two of these mutations were mapped, with half affecting cell envelope components (e.g. LPS) or biogenesis of surface structures (e.g. fimbriae, T3SS-1). Other genes required for albumen survival were related to amino acid or nucleic acid metabolism or were of unknown function. The potential importance of such factors was reinforced by a recent promoter-trap screen for *S.* Enteritidis genes that are highly induced in the chicken reproductive tract and laid eggs (Gantois *et al.* 2008*b*). This study revealed induction of stress-induced protective and reparative responses, synthesis of protein, nucleic acid and cell envelope components, and modulation of fimbrial and flagella gene expression. Phenotype microarray analyses of *S.* Enteritidis strains have provided support for the notion that an ability to metabolize certain vitamins, amino acids and fatty acids is correlated with the attainment of high cell densities, a feature that in turn is associated with reproductive tract and egg contamination (Morales *et al.* 2005). Comparison of the conservation of genes among *S.* Enteritidis isolates that differ in the degree to which they contaminate eggs partially explains the genetic basis of the observed differences (Morales *et al.* 2005). Microarray-based comparison of the gene content of serovar Enteritidis isolates collected over six decades from various hosts and locations has revealed a very high degree of similarity (Porwollik *et al.* 2005). However, subtle variation in the sequence or expression of the encoded genes may be missed by such methods.

The role of LPS in the survival of *S.* Enteritidis in egg albumen has been independently confirmed (Gantois *et al.* 2009*b*). The ability of *S.* Enteritidis to produce a high molecular mass variant of LPS has been correlated with high cell-density growth, swarm cell differentiation and a high incidence of egg

contamination (Guard-Petter 1998, 2001), and appears not to be a feature of *S.* Typhimurium strains (Parker *et al.* 2001; Guard-Bouldin *et al.* 2004). Genes putatively involved in DNA modification or repair have also been implicated in survival in albumen. The nucleases YafD and XthA (Lu *et al.* 2003) and the putative *S.* Enteritidis-specific endonuclease SEN4287 (Clavijo *et al.* 2006) are required for survival of *S.* Enteritidis in albumen and can confer improved survival upon serovar Typhimurium by an unknown mechanism.

Type I fimbriae have been implicated in adherence of *S.* Enteritidis to chicken isthmus secretions (De Buck *et al.* 2003) and egg contamination following intravenous dosing of chickens (De Buck *et al.* 2004). Immunization with purified Type I fimbriae reduces *S.* Enteritidis contamination of reproductive organs and eggs (De Buck *et al.* 2005) and candidate receptors have been identified in reproductive tract tissue (De Buck *et al.* 2003). A positive regulator of Type I fimbriae expression was also induced in the reproductive tract and in laid eggs (Gantois *et al.* 2008*b*). A role for curli fimbriae and flagella in growth in eggs has also been reported (Cogan *et al.* 2004), but the role of other surface structures, including T3SSs, in reproductive tract colonization requires further study. Recent studies have indicated that the SPI-2 regulator SsrA is required for colonization of the reproductive tract after intravenous inoculation of chickens (Bohez *et al.* 2008), however potential for pleiotropic effects exists and the role of SPI-2 genes *per se* has not been reported. Further research is also required to evaluate the role of *S.* Enteritidis-specific sequences identified by genome sequencing (Thomson *et al.* 2008). Past studies have indicated that naturally occurring *rpoS* mutants of *S.* Enteritidis which lack an alternative RNA polymerase sigma-factor are significantly impaired in their ability to colonize the oviduct of hens (Humphrey *et al.* 1998), indicating that differential regulation of shared genes may also be important.

The precise location at which *Salmonella* resides in the reproductive tract and the basis of transfer to the egg are controversial, in part owing to varying inoculation doses, routes, strains and detection methods being used in studies to date (reviewed in Gantois *et al.* 2009*a*). Some studies have indicated that reproductive tract colonization can occur in the absence of gut colonization. However, it remains a possibility that bacteria arrive at the reproductive organs by ascending infection from the common opening of the reproductive and alimentary tracts and/or by systemic translocation following passive or active uptake in the gut. Conflicting data also exist on the importance of aerosol exposure on reproductive tract and egg contamination frequency, which may be significant given the presence of *Salmonella* in faecal dust and high stocking densities used in commercial production. Ongoing studies with signature-tagged clones in the authors' laboratories aim to define the dynamics of *S.* Enteritidis infection and spread in laying hens. It has been reported that serial passage of *S.* Enteritidis through the reproductive tract, but not liver and spleen, enhances egg contamination (Gast *et al.* 2003). This implies that specific features associated with adaptation to the reproductive tract and egg environment may be positively selected, but the molecular basis of the effect is unknown.

6. MOLECULAR BASIS OF SYSTEMIC VIRULENCE

The reasons of why some *S. enterica* serovars are confined to the intestines while others translocate to distal organs are unclear. An emerging theme among pathogens associated with enteric fevers (e.g. *S. enterica* serovar Typhi (*S.* Typhi), *Brucella* spp. and enteropathogenic *Yersinia* spp.) is the use of stealth strategies to evade detection by host innate immunity (reviewed in Tsolis *et al.* 2008). In the case of *S.* Typhi, the Vi capsular polysaccharide may play a role in masking LPS from host Toll-like receptor 4. Expression of the *viaB* locus in *S.* Typhimurium impairs TLR-dependent induction of tumour necrosis factor (TNF)-α and IL-6 in murine bone marrow-derived macrophages *in vitro* as well as TNF-α and inducible nitric oxide synthase production following intraperitoneal infection of mice (Wilson *et al.* 2008). Furthermore, Vi expression in *S.* Typhimurium reduced IL-17 and GROα expression and fluid accumulation in bovine-ligated ileal loops and streptomycin-pretreated mice, whereas such responses were improved when an *S.* Typhi *viaB* mutant was tested relative to the parent strain (Raffatellu *et al.* 2007). The *viaB* locus may aid evasion of TLR-mediated detection by a second mechanism, as a regulatory gene encoded in the locus (*tviA*) can repress the transcription of the master regulators of flagella expression. Transfer of *tviA* to *S.* Typhimurium reduced secretion of the TLR5 agonist FliC and thus reduced induction of IL-8 (Winter *et al.* 2008). Such evasion may also be relevant in the pathogenesis of fowl typhoid, as strains of serovars Gallinarum and Pullurom are naturally aflagellate. Further, a mutant of *S.* Typhimurium lacking flagella induced less IL-1β mRNA and polymorphonuclear cell infiltration into the avian gut and translocation to distal organs was enhanced early after oral infection (Iqbal *et al.* 2005). Avian TLR4 may play a role in limiting systemic spread of *Salmonella* in chickens (Leveque *et al.* 2003); however, the extent to which typhoidal serovars evade detection by TLR4 in farm animals is unclear. Host species-specific usage of TLR receptor complexes has been described (Lizundia *et al.* 2008), therefore TLR evasion strategies detected in one host may not translate to another.

A further paradigm that applies to systemic versus enteric virulence of serovars is the importance of net replication (reviewed in Tierrez & García-del Portillo 2005). By analysing the segregation of a temperature-sensitive plasmid (pHSG422), differences in the net replication of *S.* Typhimurium and *S.* Choleraesuis strains were detected that may explain their differential virulence in pigs (Paulin *et al.* 2007). *S.* Typhimurium replicated more rapidly in porcine ileal mucosa, induced elevated levels of pro-inflammatory cytokines and recruited higher levels of neutrophils than *S.* Choleraesuis; whereas, the latter

persisted better in draining mesenteric lymph nodes (Paulin *et al.* 2007). Thus, rapid replication may lead to responses of a nature and magnitude that control *S.* Typhimurium locally in the intestines, whereas slow replication may aid dissemination of *S.* Choleraesuis by stealth. It is likely that differences in net growth stem from the attrition of central metabolic pathways in host-restricted and host-specific serovars as revealed by genome sequencing. However, the contribution of metabolism to virulence has received little study to date. The connection between growth rate and pathogenesis is further reinforced by the finding that *S.* Typhimurium mutants that overgrow in macrophages owing to suppression of inducible nitric oxide synthase are attenuated in mice (Eriksson *et al.* 2000). In addition to contributing to the differential virulence of serovars, one may speculate that control of net growth influences distinct phases of infection such as the acute and carrier states. It is also clear that achieving appropriate temporal and spatial control of gene expression is vital in systemic virulence. For example over-expression of SPI-2, which plays an important role in systemic pathogenesis of *S.* Dublin in calves (Bispham *et al.* 2001) and *S.* Gallinarum in chickens (Shah *et al.* 2005), has been reported to be attenuating in mice (Coombes *et al.* 2005*b*). This brings the possibility that the differential virulence of serovars may be associated with variations in the expression of a common gene set.

It is clear that both gene acquisition (e.g. *viaB*) and loss (e.g. flagella) may be associated with systemic virulence. Comparison of the genomes of serovars differing in virulence in pigs (Choleraesuis versus Typhimurium; Chiu *et al.* 2005) or chickens (Enteritidis versus Gallinarum; Thomson *et al.* 2008) reveals mutational attrition of conserved pathways and serovar-specific loci. In the absence of an annotated genome for serovar Dublin, subtractive hybridization was used to identify 24 Dublin-specific loci absent in a calf-avirulent Gallinarum strain, of which a 21 kb island played a role in intestinal colonization and systemic translocation (Pullinger *et al.* 2008). *Salmonella* virulence plasmids, and the plasmid-encoded *spv* operon in particular, influence systemic virulence of *S.* Dublin in calves but conflicting data exist on their role in enteritis (Wallis *et al.* 1995; Libby *et al.* 1997). Virulence plasmids vary markedly between serovars but their contribution to differential virulence is unclear. A further candidate that may influence systemic virulence in a serovar-specific manner is a two-partner protein secretion system (ZirST) that functions as an anti-virulence system during *S.* Typhimurium infection of mice, but appears to be defective in the sequenced serovar Choleraesuis and Gallinarum genomes (Gal-Mor *et al.* 2008).

The mechanism of translocation of typhoidal serovars from the gut to other organs in farm animals remains elusive. Studies in calves have indicated that systemic virulence of serovar Dublin compared with Gallinarum is not correlated with invasion or enteritis, but with persistence in ileal mucosa and associated lymph nodes and translocation *via* efferent lymphatics (Paulin *et al.* 2002). Relatively few bacteria were detected in the blood early after inoculation of the bovine intestines with *S.* Dublin and studies using signature-tagged clones indicate that the bacteria arriving at the liver and spleen are the same ones in the same proportions as represented in the lymph (Pullinger *et al.* 2007, 2008). This indicates that translocation *via* the lymphatic system may be relevant in typhoidal disease in cattle, in contrast to observations with *Y. pseudotuberculosis* in mice (Barnes *et al.* 2006). These studies also indicated that T3SS-2, despite being important for intestinal colonization after oral infection and systemic virulence following intravenous dosing (Bispham *et al.* 2001), is dispensable for early translocation (12–24 h) from the gut to the liver and spleen (Pullinger *et al.* 2007). It is therefore open to question if the role of T3SS-2 in intracellular survival is relevant in the early stages of spread. Extracellular lymphatic translocation has also been described for *S.* Abortusovis in an ovine lymph duct cannulation model following injection of a vaccine strain into oral mucosa (Bonneau *et al.* 2006). Systemic translocation of *S.* Dublin *via* ileal lymphatics in calves was dependent on T3SS-1 implying that active invasion, as opposed to passive uptake by antigen-sampling cells, is important in contrast to some observations in mice (Pullinger *et al.* 2007). It is a widely held view that translocation of *Salmonella* from the subepithelial space to distal sites occurs in macrophages, and support exists for a role for CD18-positive phagocytes in this process in mice (Vazquez-Torres *et al.* 1999). In cattle, *S.* Dublin transits rapidly through the epithelial layer and associates with MHC class II-positive cells in the lamina propria (Pullinger *et al.* 2007). However, it remains unclear how it arrives at draining nodes or escapes into an extracellular niche in efferent lymph. Numerous virulence-associated genes (Pullinger *et al.* 2007), serovar-specific genes (Pullinger *et al.* 2008) and two-component sensory systems (M. Stevens, in preparation) have so far been found to be dispensable for early translocation of serovar Dublin in calves.

7. IMPACT OF STRESS ON *SALMONELLA* PATHOGENESIS AND TRANSMISSION

Transport and social stress are correlated with increased faecal excretion of *S.* Typhimurium in pigs and reactivation of asymptomatic infection (Isaacson *et al.* 1999; Callaway *et al.* 2006). Transportation also enhances hide and faecal contamination of beef cattle with *Salmonella* (Barham *et al.* 2002), and stress at the onset of laying and depopulation ('thinning') of poultry flocks is believed to contribute to avian salmonellosis and zoonotic transmission (reviewed in Humphrey 2006). Such phenomena may be partially explained by the ability of *Salmonella* to respond to host stress-related catecholamines by activating growth and virulence gene expression. Noradrenaline (NA), which is released by the enteric nervous system under stress, promotes the virulence of *S.* Typhimurium in mice (Williams *et al.* 2006), *S.* Enteritidis in chicks (Methner *et al.* 2008) and *Salmonella* encephalopathy in calves (McCuddin *et al.* 2008). One hypothesis is that NA facilitates bacterial growth by mediating the release of iron

from host iron-storage proteins in a manner dependent on breakdown products of the bacterial siderophores enterobacterin and salmochelin B4 (Methner *et al.* 2008). An alternative hypothesis, not exclusive to that above, is that *Salmonella* senses and responds to NA using an orthologue of the *Escherichia coli* O157 : H7 adrenergic sensor kinase QseC (also known as PreB, YgiY and STM3178 in *S.* Typhimurium). *Escherichia coli* O157 : H7 QseC is an inner membrane sensor kinase that autophosphorylates on binding NA or adrenaline, then transfers the phosphate moiety to its cognate response-regulator QseB, thereby modulating the transcription of genes under its control (Clarke *et al.* 2006). It is not yet known if *S.* Typhimurium QseC binds NA and activates QseB as a consequence. However, QseC is required for invasion of IPEC-J2 porcine jejunal cells, intestinal colonization of swine (Bearson & Bearson 2008) and systemic virulence in mice (Merighi *et al.* 2009). An inhibitor of *E. coli* O157 : H7 QseC signalling (LED209) also impairs systemic virulence of *Salmonella* in a murine model (Rasko *et al.* 2008). Two-component sensory systems are known to integrate multiple signals and it remains to be proven that the QseBC system is an essential link in the response of *Salmonella* to stress-related catecholamines *in vivo*. NA has been reported to activate expression of the *S.* Typhimurium T3SS-related *sifA* gene in a manner sensitive to LED209 and mutation of *qseC* (Rasko *et al.* 2008). However, recent microarray studies of the QseBC regulon (Merighi *et al.* 2009) and the response to adrenaline (Karavolos *et al.* 2008) do not support effects on T3SS loci. Recent data indicate that NA augments *S.* Typhimurium-induced enteritis in bovine-ligated ileal loops in a manner independent of QseC and other candidate adrenergic sensors (M. Stevens, in preparation). It has been reported that NA modulates transcription of *S.* Typhimurium flagellar genes and motility (Bearson & Bearson 2008), which may be significant given the role of *flhD* in enteritis (Schmitt *et al.* 2001) and the fact that *fljB* mutants were negatively selected by STM (Morgan *et al.* 2004). However, conflicting reports exist on the magnitude of the effect of catecholamines on flagella regulation in *S.* Typhimurium and the requirement for QseC in this process (Karavolos *et al.* 2008; Merighi *et al.* 2009).

Irrespective of the molecular mechanisms at play, it is clear that stress has the potential to augment *Salmonella* virulence and increase pathogen entry into the food chain and environment. It is likely that such phenomena involve modulation of both pathogen and host processes. Indeed, stress at the onset of sexual maturity in laying hens may be correlated with increased translocation of *S.* Pullorum to the reproductive tract and egg in a manner associated with suppression of T cell-mediated immunity (Wigley *et al.* 2005). It remains to be seen if the same applies to *S.* Enteritidis, but it is tempting to speculate that stress associated with the onset of laying is correlated with the temporal clustering of *S.* Enteritidis-positive eggs laid at this time (Humphrey *et al.* 1989).

8. IMPACT OF HOST GENETIC BACKGROUND

Variation in resistance to salmonellosis has been reported within and between breeds of livestock. In inbred lines of poultry, heritable differences in resistance to both systemic (Bumstead & Barrow 1993) and enteric (Duchet-Suchaux *et al.* 1997) *S. enterica* infections exist and attempts have been made to correlate these with the nature, location, magnitude and timing of host-innate responses (reviewed in Wigley 2004). Resistance to avian systemic salmonellosis in inbred lines is partly mediated by a single dominant locus on chicken chromosome 5 (*SAL1*; Mariani *et al.* 2001) and recent studies have refined the region associated with resistance to 14 candidate loci (Fife *et al.* in press). Among the variable genes in the *SAL1* loci of resistant and susceptible lines is the chicken orthologue of Akt1, a cellular kinase that plays a role in controlling intracellular replication of *Salmonella* (Kuijl *et al.* 2007). Polymorphisms affecting *NRAMP1* (*Slc11a1*) and *TLR4* have also been implicated in resistance to systemic disease. Resistance to intestinal colonization of poultry by *Salmonella* is believed to be determined by multiple genes and is less well understood. Characterization of a resource population of pigs exhibiting heritable differences in resistance to systemic *S.* Choleraesuis infection revealed that resistant pigs had higher levels of circulating neutrophils with improved phagocytic and oxidative function, yet mitogen-induced proliferation of lymphocytes was impaired (van Diemen *et al.* 2002). The underlying genetic basis of these effects is unknown. Differences in susceptibility of cattle breeds to salmonellosis exist, for example Friesian calves are more resistant to *S.* Typhimurium infection than Jersey calves (Wray & Sojka 1978), but genetic linkage or correlates of resistance are yet to be described.

9. IMPACT OF INTESTINAL MICROFLORA ON *SALMONELLA* PATHOGENESIS

It has long been recognized that possession of a developed intestinal microflora affords resistance to enteric bacterial infections; however, the extent to which this is exerted by competitive exclusion or by expansion of immune repertoire and function is open to question (reviewed in Stecher & Hardt 2008). Depletion of intestinal microflora with streptomycin is necessary for *Salmonella*-induced enteritis in mice (Barthel *et al.* 2003) and the development of a 'super-shedder' phenotype and efficient transmission (Lawley *et al.* 2008). Preparations of intestinal microflora are widely used in commercial broiler production and some have been confirmed to afford protection against *Salmonella* experimentally (Nakamura *et al.* 2002). The dynamic role of the flora in modulating the outcome of *Salmonella*–host interactions is becoming clear. For example, a common intestinal symbiont of the genus *Bacteroides* was reported to attenuate the inflammatory response of mice to *Salmonella* infection (Kelly *et al.* 2004) and recent studies have indicated that this may be owing to the ability of a surface polysaccharide to modulate host immunity towards symbiosis (Mazmanian *et al.* 2008). Conversely, it

has been reported that *Salmonella* infection induces transient shifts in the composition of the microflora and that this may be a strategy deployed by the pathogen to target competitors (Stecher *et al.* 2007). An *invG sseD* double mutant incapable of inducing inflammation was outcompeted by the microflora in normal mice, but this effect was not seen in mice with inflammatory bowel disease (Stecher *et al.* 2007). Barman *et al.* (2008) separately reported that SPI-2, but not SPI-1, is required for microflora shifts in mice, even though both were required for inflammation and persistence. Recent studies have indicated that motility and chemotaxis are required for net replication of *S.* Typhimurium in the inflamed intestines, but not the normal gut in mice (Stecher *et al.* 2008). In part, this appears to be a consequence of utilization of high-energy nutrients released during the host response and implies that *Salmonella* may have evolved strategies to take advantage of the host environment following inflammation. Such studies indicate a need to evaluate the role of *Salmonella* genes in the face of the host response, as well as in naive experimental animals. The extent to which events in murine models hold true in the complex and distinct intestinal environments of ruminants, pigs and poultry requires investigation.

10. CONCLUDING REMARKS

For *S. enterica* strains found in food-producing animals to cause diarrhoeal disease in humans, they must colonize the intestines or reproductive tract of the reservoir host, survive in the food chain and environment and cross the species divide to colonize the human intestines and elicit enteritis. While this review highlights some of the molecular mechanisms associated with *Salmonella* persistence and pathology in livestock, the molecular basis of transmission to humans remains relatively ill-defined. Analysis of available genome sequences implies that the broad host range of foodborne serovars is correlated with the maintenance of a wider repertoire of intact genes than may be found in host-restricted or host-specific variants; however, the precise traits associated with zoonotic and epidemic potential are unknown. Identification of such traits is vital to assess the risk posed to humans by *S. enterica* strains found in animals and for effective targeting of intervention strategies. The advent of next-generation sequencing can be expected to yield new insights in this area, particularly if applied to epidemic variants and hitherto unfashionable serovars that are frequently found in animals yet rarely appear in humans. It is also becoming clear that the outcome of infection of farm animals, and thus potential for transmission, is contingent on a plethora of host parameters including host genetic and immune status, stress and the intestinal microflora. In this regard, the outcome of infection of farm animals and potential for spread is not predetermined, but reflects the interaction of multiple host and pathogen processes. Analysis of the differential virulence of *S. enterica* serovars in animals provides powerful models to study the basis of naturally occurring enteric and systemic disease. Such studies are vital to the rational development of control methods and indicate that both conserved and host-specific factors are deployed by *Salmonella*. A need therefore exists to confirm observations in rodent or cell-based models in target animals where feasible.

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